

Response of five tree species to salinity and waterlogging: shoot and root biomass and relationships with leaf and root ion concentrations

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Abstract

Unproductive saline and waterlogged wastelands could be beneficially transformed into agroforestry systems using trees tolerant to these stresses. We studied the salinity and waterlogging tolerance of five Australian tree species (*Acacia salicina*, *Casuarina glauca*, *Casuarina obesa*, *Eucalyptus camaldulensis* and *Eucalyptus occidentalis*) during seedling stage and their relationships with root and leaf ion concentrations. Eight-month old plants were exposed for five months to five irrigation water salinity treatments (EC values between 2 and 22 dS m⁻¹) and two waterlogging treatments (drained or waterlogged). The salinity tolerance of the five species was high, although decreased in waterlogged conditions. Irrespective of salinity, the two *Casuarina* species were more tolerant and the other three species were less tolerant to waterlogged than drained conditions. In all species, salinity and waterlogging increased leaf Cl⁻ and Na⁺ and decreased leaf Ca²⁺, but not leaf K⁺. Root Cl⁻ and Na⁺ increased with salinity but not with waterlogging. Salinity tolerance was negatively correlated with Cl⁻ and Na⁺ leaf accumulation rates per unit increase in salinity. Waterlogging reduced the ability of the seedlings to exclude Cl⁻ and Na⁺ from the leaves. The two most salt tolerant *Casuarina* species under both drained and waterlogged conditions showed the highest leaf Cl⁻ and Na⁺ exclusion and the highest root Cl⁻ and Na⁺ accumulation, suggesting that sequestration of these toxic ions in their roots was a significant salt-tolerant mechanism. Revegetation of saline and waterlogged wastelands with these tolerant *Casuarina* species could be profitably used for biomass, biofuel and renewable energy production.

Keywords: Agroforestry, Aerial biomass, Root biomass, *Acacia*, *Casuarina*, *Eucalyptus*

Introduction

The world's population will expand to about eight billion people in 2025, with concomitant needs to increase production of food, non-food and other agro-outputs. These rising needs will require, besides agricultural intensification, its expansion into presently unproductive salt and waterlogged areas. Agroforestry, an integrated approach that combines agricultural and forestry technologies, could be applied to these wastelands using trees, shrubs and crops tolerant of these stresses (Marcar and Crawford 2004; Turner and Ward 2002; Singh et al., 1994). The benefits of these "biosaline agroforestry plantations" will include a diversified income stream from biomass, biofuel and renewable energy production, an improved habitat for wildlife and humans, and the atmospheric nitrogen fixation by some species that could provide part of the nitrogen required for production of other species. These plantations could as well contribute to site rehabilitation, lowering of water tables and reduce salt loads to streams (Feikema and Baker 2011), although several studies cited by Thorburn (1996) have questioned the long-term sustainability of this revegetation strategy for water table control in saline areas due to a progressive salinity increase in the upper part of the soil profile. Therefore, the success of these agroforestry systems relies to a large extent on the assessment of the salinity and waterlogging tolerance of the cultivated species.

The general effects of salinity on plant growth are well documented (Munns and Tester 2008), but the effects of waterlogging and the interactions between salinity and waterlogging are not fully understood. Waterlogging becomes an additional constraint to plant growth in saline areas because, besides the osmotic and the Na^+ or Cl^- toxic effects derived from the accumulation of salts, the soil becomes oxygen deficient (hypoxic) (Barrett-Lennard and Shabala, 2013). The main effects of hypoxic conditions on plants are the reduction of ATP formation, the lower growth of roots and root survival, the lower uptake of nutrients, and the reduction in the capacity of plants to exclude toxic ions like Na^+ and Cl^- from the shoots (Kozłowski 1997; Barrett-Lennard 2003; Barrett-Lennard and Shabala, 2013). This ion-exclusion reduction could exacerbate plant damage under saline conditions since there is considerable evidence that differences in the ability to exclude these ions is one of the most important factors underlying intraspecific differences in tolerance (Allen et al. 1994; Pessarakli 1999; Carter et al. 2006).

Van der Moezel et al. (1988) analyzed the seedling performance of seven Australian tree species subject to salinity/waterlogging conditions and concluded that the species with the highest tolerance to non-saline waterlogging were also more tolerant of saline-waterlogging conditions. Aswathappa and Bachelard (1986) found that the more salt-tolerant *Casuarina* species accumulated less Na^+ and Cl^- in their shoots and maintained a higher selectivity of K^+ over Na^+ than less tolerant species. Furthermore, root Na^+ and Cl^- concentrations in salt tolerant *Casuarina* species were either similar to or lower than those in moderately tolerant *Casuarina* species. Craig et al. (1990) found that the slowest rates of growth in ten *Acacias* subject to salt stress were associated with the highest Na^+ concentrations in the uppermost

phylloides. Chen et al. (2002) indicated that differences in salt tolerance of three poplar genotypes depended on their ability to restrict salt transport from roots to leaves, and Fung et al. (1998) concluded that the more tolerant poplar clones had consistently lower shoot Na^+ contents and $\text{Na}^+:\text{K}^+$ ratios than the less tolerant clones. Pessarakli (1999) indicated that most plants subject to salt stress have generally higher root and lower leaf Na^+ and Cl^- concentrations, and that many salt-susceptible species have higher leaf Cl^- than Na^+ , whereas many salt-tolerant species have higher leaf Na^+ than Cl^- . Kozłowski (1997) concluded that combined flooding and salinity typically decreased growth and survival of woody plants more than did either stress alone. Zalesny et al. (2008) indicated that across the eight *Populus* genotypes studied, Na^+ levels were greatest in the roots and Cl^- levels greatest in the leaves.

For the species studied in this work (*Acacia*, *Casuarina* and *Eucalyptus*), *A. salicina* was ranked as highly tolerant to salinity [groundwater EC = 5-15 dS/m (BOSTID 1990); threshold E_{Ce} = 15-40 (Marcar et al. 1993) and 8-16 dS/m (Marcar et al. 1999)] and able to tolerate combinations of waterlogging with moderate salinity (Marcar et al. 1993); *C. glauca* was ranked as highly tolerant to salinity [threshold E_{Ce} = 15-40 (Marcar et al. 1993) and 8-16 dS/m (Marcar et al. 1999; Ansari et al. 1999) and low tolerant to waterlogging (Marcar et al. 1999); *C. obesa* was considered to be more tolerant than *C. glauca* by Marcar et al. (1999); *E. camaldulensis* was ranked for salinity as moderately tolerant by Marcar et al. (1999) (threshold E_{Ce} = 4-16 dS/m) and slightly tolerant by Benyon et al. (1999) (threshold E_{Ce} = 2 dS/m) and Ansari et al. (1999). Marcar et al. (1993, 1999) ranked this species as having low tolerance to waterlogging, whereas Ansari et al. (1999) ranked it as highly tolerant. *E. occidentalis* was ranked as more tolerant to salinity than *E. camaldulensis* [threshold E_{Ce} = 10 dS/m (Benyon et al. 1999) and 8-16 dS/m (Marcar et al. 1999)], and with a similar and low tolerance by Ansari et al. (1999). Marcar et al. (1993) indicated that this species had a low tolerance to waterlogging (lower than that for *E. camaldulensis*). Thus, contrasting results and with large threshold E_{Ce} ranges were obtained in different studies, showing that there are inadequate field data to quantify accurately the salinity and waterlogging tolerance of these species.

The objectives of the present study were to establish in five Australian tree species (1) the salinity tolerance under drained and waterlogging conditions, (2) the waterlogging tolerance, and (3) the relationships of Na^+ , Cl^- , K^+ and Ca^{2+} accumulation in leaves and roots to salinity and waterlogging tolerance. The accomplishment of these objectives will enhance the expansion of agroforestry systems cultivated with the most adequate species for high salinity and waterlogged landscapes.

Materials and methods

Plant material

Seeds from five Australian native tree species were selected from the collection available in the Australian Tree Seed Centre (ATSC, CSIRO, Canberra). The species and provenances were: *Acacia salicina* Bark. (seedlot n°18484, Lake Buchanan, QLD), *Casuarina*

glauca Sieb. (seedlot n°15923, Tuross lake, NSW), *Casuarina obesa* Miq. (seedlot n°15396, Dumbleyung lake, WA), *Eucalyptus camaldulensis* Dehn. var. *camaldulensis* (seedlot n°20561, Lake Albacutya, VIC), and *Eucalyptus occidentalis* Endl. (seedlot n°15385, Bremer Bay, WA). These vigorous, evergreen and long-lived trees were selected because of their reported moderate to high tolerance to salinity and/or waterlogging, and their production of durable wood that may be used in biosaline agroforestry for furniture, biomass, biofuel and renewable energy manufacturing (Marcar and Crawford 2004). Some of the studied species (*Casuarina* and *Acacia*) present nodulation in their original habitats with symbiotic organisms from genus *Frankia* and *Rhizobium*, respectively

Experimental design and growing conditions

The experiment was carried out during 2009 in the Agrifood Research and Technology Centre of Aragón (CITA) located in the Middle Ebro River Basin (Zaragoza, Spain, 0°49'W, 41°44'N). The mean air temperature and total precipitation from early May to late October 2009 were 21.4°C and 84.8 mm, respectively, and the potential evapotranspiration (ET_0) was 882 mm.

The experimental design included the five tree species indicated, five irrigation water salinity treatments ($EC_{iw} = 2, 7, 12, 17$ and 22 dS m^{-1}), two waterlogging treatments (no water table (NWT) or drained conditions, and shallow water table (WT) or waterlogged conditions) and six replicates with one plant per replicate, for a total of 300 plants.

Seeds of *Acacia salicina* required a pre-treatment of 20-min immersion in a solution of sulphuric acid (50%) for appropriate germination. The other species did not require pre-treatments. The seeds were germinated in 25-cm high pots filled with a mixed substratum of peat and sand. The plants were grown in a greenhouse at a constant temperature of 25-30°C until they were eight-months old. On 5 May 2009, the plants were transplanted into 40-L plastic, white pots (36 cm in diameter and 40 cm height) filled with 1-2 mm diameter siliceous sand with a 21% volumetric water holding capacity. About 80 g of a controlled-release fertilizer (Osmocote®, SCOTTS) with 15-9-9-3% of N-P-K-MgO and micronutrients were applied to each pot before transplanting. An additional 30 g fertilizer per pot was applied in August 17 to ensure an adequate nutrient availability during the entire experiment.

Each pot was drip-irrigated using two 8 L h^{-1} emitters. Each salinity sector was equipped with an automatic valve controlled by an irrigation controller (Progrés S.A., Lleida, Spain) connected to SECTOR® equipment (ITC S.L., Barcelona, Spain). This equipment regulated the EC of the water applied to each target salinity treatment through a variable frequency drive connected to an injection pump. A 300 g L^{-1} NaCl solution was injected at the appropriate rate to the irrigation water supply ($EC_{iw} = 2 \text{ dS m}^{-1}$) to obtain the target irrigation water salinity treatments. NaCl was used because these ions are dominant in the major salt-affected areas of the world. The Ca^{2+} levels in the water supply (about 8 meq L^{-1} , Table 1) were sufficient to prevent ion imbalances typical in experiments with pure NaCl solutions.

The pots in the drained treatment (NWT) had a hole at their bottoms connected to a plastic tube for the free drainage of the soil solution. The pots in the waterlogged treatment (WT) had a permanent water table located at 10 cm from the soil surface established by lifting the plastic outlets to this height. Although the soil surface in the WT treatment remained close to saturation at all times, the imposed hypoxic stress did not produce the death of plants.

The plants were irrigated with the canal irrigation water under free-draining conditions for about one month after transplanting, and the salinity and waterlogging treatments were initiated on June 9, when all plants appeared uniform and were adapted to the external conditions. The salt concentrations of the water applied in the different saline treatments were increased linearly during 10 days to allow plant acclimation to salt stress.

A total of 58 irrigations were given during the experiment, each consisting of three to five 10-min applications per day, depending on potential ET demand, separated by about eight to five hours. This sequence kept the soil continuously near field capacity. A high leaching fraction close to 100% was imposed to attain a salinity of drainage waters similar to that of the corresponding irrigation waters (i.e., steady-state soil solution EC).

Salinity of irrigation (EC_{iw}) and drainage (EC_{dw}) waters

A sample of irrigation water in each irrigation event and salinity treatment was collected and the EC_{iw} was measured with a calibrated portable conductivity meter. Similarly, the EC_{dw} of the pooled drainage waters collected from the six replicated pots of each species, salinity and waterlogging treatment was measured after each irrigation event. The ionic composition of the irrigation and drainage waters were measured three times during the experiment.

For each irrigation event, the EC of the soil solution (EC_{ss}) was calculated as the average of EC_{iw} and EC_{dw}. The time-average EC_{ss} along the duration of the experiment characterized the salinity stress at which each species was subject in each salinity and waterlogging treatment.

Plant biomass

The aerial part of each plant was harvested on October 27, twenty weeks after the start of the saline and waterlogging treatments. Each plant was oven-dried at 65°C until constant weight. The total shoot biomass was expressed as kilograms of the shoot dry matter (SDM) per plant.

The roots were extracted from the containers at the end of the experiment, allowed to dry in the air and detached from the soil matrix. In some species, a significant portion of the roots was lost when trying to separate them from the soil, whereas in other species a significant amount of soil was held within the finer roots and we could not separate entirely the roots from the soil. Therefore, the weight of the total root biomass expressed as kilograms of the root dry matter (RDM) per plant should be taken with caution due to these problems.

Leaf and root ion concentrations

A representative sample of leaves from different middle-branches of each live tree was collected from all treatments a few days before harvesting. A representative sample of roots was also collected in four replications of the 2 and 17 dS m⁻¹ NWT and WT treatments. The leaves and roots were oven-dried at 65°C until constant weight and were finely ground using a 0.5-mm sieving mill. A 0.25 g sample was extracted with 50 mL of an extracting solution made up of 900 mL deionized water, 100 mL acetic acid, and 6.4 mL nitric acid. Chloride was analyzed by the Cotlove (1963) coulometric-amperometric titration procedure, and sodium, potassium and calcium by flame photometry. Ion concentrations (Cl⁻, Na⁺, Ca²⁺, and K⁺) were expressed as meq kg⁻¹ of total dry matter.

Statistical analysis

The statistical analysis were performed using the SAS[®] 9.1 software. The analysis of variance of shoot dry matter was performed with the Proc General Linear Model (GLM) and multiple comparisons were performed using the Tukey test at $P = 0.05$. The relative salinity tolerance of each species was obtained using the sigmoidal-shape model proposed by Steppuhn et al. (2005):

$$Yr = \frac{1}{1 + \left(\frac{ECss}{ECss_{50}} \right)^{e^{(s \cdot ECss_{50})}}}$$

where Yr is the shoot dry matter (SDM) relative to that in the control treatment (NWT- 2 dS m⁻¹), ECss is the time-average soil solution EC, ECss₅₀ is the ECss at which SDM decreases by 50% over the control, and s is an empirical parameter associated to the shape of the adjusted curve. The model was fitted to each species and salinity-waterlogging treatment through the NLIN procedure of the SAS[®] 9.1 software.

Results

Irrigation water (ECiw), drainage water (ECdw) and soil solution (ECss) salinity

Table 1 summarizes the average \pm standard deviation ECiw and ECss of the 58 samples collected along the 2009 experimental year in each saline treatment. No distinctions were made between the NWT and WT treatments because they were not different ($P > 0.05$). The ECss values were close to the corresponding target saline treatments (Table 1), showing that the experimental procedure was reliable. Irrigation water Na⁺, Cl⁻ and sodium adsorption ratio (SAR) increased with increases in ECiw, but the Na⁺/Cl⁻ ratio remained close to one in all treatments because NaCl was the salt added for increasing ECiw. ECdw was about 10% higher than ECiw in each saline treatment due to plant ET. ECss values were therefore considered to be the best estimates of salinity stress at which the trees were subjected.

Plant survival under saline and waterlogging treatments

None of the 120 *Casuarina* plants showed leaf necrosis or were severely affected or dead, irrespective of the imposed salinity and waterlogging treatments. Thus, these two species appear to be the most tolerant of the five studied species in terms of their ability to survive under these stresses.

The NWT (drained) treatment did not severely affect the species at any salinity treatment, except with *A. salicina* with one and two trees out of six affected at the 17 and 22 dS m⁻¹ salinity treatments, respectively. In contrast, the WT (waterlogged) treatment had a severe negative effect on some species that increased with increasing salinity. Thus, with waterlogging and the highest salinity treatment (22 dS m⁻¹), *A. salicina* had three trees that were severely affected and three trees that were dead, and *E. camaldulensis* had six trees dead at the end of the experiment. *E. occidentalis* was the most sensitive species to waterlogging, since irrespective of the salinity treatment at least four out of six trees were severely affected or dead.

Shoot dry matter (SDM) under saline and waterlogging treatments

The analysis of variance of SDM indicates differences ($P < 0.0001$) between tree species, salinity and waterlogging treatments, and the interactions “species*salinity” and “species*waterlogging”.

SDM of the five species decreased ($P < 0.01$) with increases in salinity under both drained (NWT) and waterlogged (WT) treatments (Fig. 1). SDM differences between species were highest at the lowest salinity treatment and decreased as salinity increased, particularly in the NWT treatment. Thus, the SDM in the NWT-2 dS m⁻¹ treatment ranged between 0.38-0.93 kg plant⁻¹, whereas in the NWT-22 dS m⁻¹ treatment the range was only between 0.22-0.34 kg plant⁻¹.

In the NWT treatment (Fig. 1a), *A. salicina* and the two *Casuarina* species had in general lower ($P < 0.05$) SDM values in all salinity treatments than the two *Eucalyptus* species. In the WT treatment (Fig. 1b), SDM of the two *Casuarina* species were higher ($P < 0.05$) in all salinity treatments than SDM of *Acacia*. SDM of *E. occidentalis* was higher ($P < 0.05$) than SDM of *Acacia* at $EC \leq 12$ dS m⁻¹, but they were not different ($P > 0.05$) at $EC \geq 17$ dS m⁻¹. SDM of *E. camaldulensis* was very high in the WT-2 dS m⁻¹ treatment, but decreased sharply with increases in salinity so that at the highest salinity treatment it was among the lowest of all tested species.

Irrespective of the salinity level, *Acacia* and the two *Eucalyptus* species had higher SDM under drained (NWT) than under waterlogged (WT) conditions. Thus, the ratios of the means of the five salinity treatments in NWT and WT conditions (mean SDM_{NWT}/mean SDM_{WT}) were 2.0 (*E. occidentalis*), 2.3 (*E. camaldulensis*) and 3.6 (*A. salicina*). In contrast, the two *Casuarina* species had lower SDM under drained (NWT) than under waterlogged (WT) conditions (mean SDM_{NWT}/mean SDM_{WT} = 0.6 in *C. glauca* and 0.8 in *C. obesa*).

Salinity tolerance of shoot dry matter (SDM) under drained and waterlogged conditions

Figure 2 shows the relationships between relative shoot dry matter (RSDM, where RSDM = 1 corresponds to the SDM measured in the NWT-2 dS m⁻¹ control treatment of each species) and average soil solution salinity (ECss) for each tree species subject to drained (NWT) and waterlogged (WT) treatments. In general, the standard errors of the means were low, showing that the six replicated trees of each saline and waterlogging treatment had in most cases a similar response.

In agreement with the SDM values given in Fig. 1, the RSDM values in the 2 dS m⁻¹-WT treatment were lower than one in *A. salicina* and the two *Eucalyptus* species, and higher than one in the two *Casuarina* species. In all species and WT treatments, RSDM decreased with increases in ECss, but the shape of the decline was different, with a threshold-slope trend in *C. obesa* and *E. occidentalis* and an almost continuous and decreasing trend in the other species. All the sigmoidal models had R² values greater than 0.81 indicating a good fit of the observations to the proposed model.

In terms of relative shoot dry matter (Fig. 2), the species were more tolerant to salinity (i.e., higher ECss₅₀ values) in the drained than in the waterlogged treatments, except with the two *Casuarina* species where the ECss₅₀ values were quite similar. Thus, the species-average ECss₅₀ in the drained treatment (19.0 dS m⁻¹) was 21 % higher than in the waterlogged treatment (15.7 dS m⁻¹). These differences in ECss₅₀ were much higher in some species than in others: 4.5 dS m⁻¹ in *E. occidentalis*, 3.6 dS m⁻¹ in *E. camaldulensis*, and 3.1 dS m⁻¹ in *A. salicina*.

The data in Figures 1 (SDM in absolute terms) and 2 (SDM in relative terms) show that for the range of salinities studied the two *Casuarina* species were more tolerant and the other three species were less tolerant to waterlogged than drained conditions. Based on the ECss₅₀ estimates shown in Fig. 2, the ranking in salinity tolerance of the five tested species in drained (NWT) and waterlogged (WT) conditions was:

NWT: *E. camaldulensis* < *A. salicina* < *E. occidentalis* = *C. glauca* = *C. obesa*

WT: *E. camaldulensis* < *A. salicina* < *E. occidentalis* < *C. glauca* = *C. obesa*

Thus, four different qualitative groups could be established in terms of their relative salinity tolerance: very high (the two *Casuarina* species), high (*E. occidentalis*), low (*A. salicina*), and very low (*E. camaldulensis*). Overall, of the five species tested the two *Casuarina* species showed the highest tolerance to both salinity and waterlogging.

Root dry matter (RDM) under saline and waterlogging treatments

The previously indicated methodological problems have not allowed for a sound statistical analysis of data. Therefore, the RDM results should be taken from a qualitative point of view, and an assessment of the salinity tolerance of RDM as that performed with SDM was not attempted.

RDM of the five species tended to decrease with increases in salinity (ECss) under both drained (NWT) and waterlogged (WT) conditions (Fig. 3). For a given species, the highest

difference between the NWT and WT treatments was generally observed at the lowest salinity, whereas RDM in NWT and WT were similar at high salinity values.

Two distinct behaviours were observed between the two *Casuarina* species where RDM was higher (or similar) in the waterlogged than in the drained treatment, and the other three species where RDM was lower or similar in the waterlogged than in the drained treatment. Thus, the ratios mean RDM_{NWT}/mean RDM_{WT} were 0.4 (*C. obesa*), 0.6 (*C. glauca*), 3.3 (*E. camaldulensis*), 3.4 (*E. occidentalis*) and 7.2 (*A. salicina*). These ratios indicate that the two more tolerant *Casuarina* species to waterlogging produced more roots in this treatment than in the drained treatment, whereas the three more sensitive species to waterlogging produced less roots in this treatment than in the drained treatment.

Leaf ion concentrations (Cl^- , Na^+ , Ca^{2+} , K^+)

Leaf Cl^- increased ($P < 0.05$) with increased ECss in all species and waterlogging treatments, except in the drained treatment (NWT) of the two *Casuarina* species (Fig. 4). Leaf Cl^- was relatively low (close to 100 meq kg^{-1}) in some species subject to the lowest ECss, and increased to values above 2000 meq kg^{-1} in some species (*E. camaldulensis* and *A. salicina*) subjected to the highest waterlogged-ECss treatment. Although the slope of *C. obesa* under drained conditions was not significant ($P > 0.05$), its leaf Cl^- at the highest ECss was almost two times higher than at the lower ECss.

The slopes of the linear regressions of leaf Cl^- vs. ECss were higher ($P < 0.05$) in the waterlogged (WT) than in the drained (NWT) treatment in all species (Fig. 4). The WT slopes were between 2.2 (*A. salicina*) and 5.2 (*E. camaldulensis*) times higher than the NWT slopes, indicating that the rate of leaf Cl^- accumulation per unit increase in ECss was much higher under waterlogged than under drained conditions.

The slopes of the linear regressions of leaf Cl^- vs. ECss varied between tree species (Table 2), ranging in the drained treatment from zero in the two *Casuarina* species to $38.5 \text{ meq kg}^{-1}/\text{dS m}^{-1}$ in *A. salicina*. The slopes of the two *Eucalyptus* species were quite similar and lower ($P < 0.05$) than the slope of *A. salicina*. According to these slopes, three different groups of species were found to be different ($P < 0.05$), with a ranking somewhat different in the NWT and WT treatments:

NWT: *A. salicina* > *E. camaldulensis* = *E. occidentalis* > *C. obesa* = *C. glauca*

WT: *E. camaldulensis* = *A. salicina* > *E. occidentalis* = *C. obesa* > *C. glauca*

The trends of leaf Na^+ and Cl^- concentrations were generally similar. Leaf Na^+ increased ($P < 0.05$) with increased ECss in all species and waterlogging treatments, ranging from the lowest value (55 meq kg^{-1}) in the NWT-2 dS m^{-1} treatment of *A. salicina* to the highest value (close to 2000 meq kg^{-1}) in the WT-22 dS m^{-1} treatment of this species (Fig. 5). All the species had higher slopes ($P < 0.05$) in the WT than in the NWT treatment. However, the ratio between the slopes in the WT and NWT treatments was species-dependent, ranging between 1.9 (*A. salicina*) and 4.3 (*E. camaldulensis*). Thus, as for Cl^- , the rate of leaf Na^+ accumulation per unit increase in ECss was much higher under waterlogged than under drained conditions.

The slopes of the linear regressions of leaf Na^+ vs. ECss varied between tree species, ranging from 7.0 (*C. glauca*) to 48.4 $\text{meq kg}^{-1}/\text{dS m}^{-1}$ (*A. salicina*) in the drained (NWT) treatment, and from 18.6 (*C. glauca*) to 94.1 $\text{meq kg}^{-1}/\text{dS m}^{-1}$ (*A. salicina*) in the waterlogged (WT) treatment (Table 2). According to these slopes, three groups of species were found to be different ($P < 0.05$), with a ranking almost similar in the NWT and WT treatments:

$$A. salicina \geq E. camaldulensis \geq E. occidentalis = C. obesa > C. glauca.$$

Leaf Ca^{2+} decreased ($P < 0.1$) with salinity increases in eight out of the ten species-waterlogging combinations (Table 2). *A. salicina* had the lowest slope of all species in both NWT and WT treatments, with significant differences with the rest of species in the NWT treatment, and with *E. occidentalis* in the WT treatment. These results show that leaf Ca^{2+} decreased with increases in salinity, and that the rates of decrease per unit increase in ECss were similar in NWT and WT in all species except *A. salicina*. Comparing across the five species, no correlations ($P > 0.05$) were found between leaf Ca^{2+} decreases and leaf Na^+ or Cl^- increases with salinity.

Leaf K^+ was not affected ($P > 0.05$) by salinity and waterlogging, and none of the relationships between leaf K^+ and ECss were significant (data not given). A summary of leaf K^+ concentrations at three salinity treatments (2, 12 and 22 dS m^{-1}) is presented in Table 3. Of the five species examined, only *C. obesa* and *C. glauca* had, averaging over the five salinity treatments, lower leaf K^+ in waterlogged (WT) than in drained (NWT) conditions. Leaf K^+ was not correlated ($P > 0.05$) with leaf Na^+ at any salinity and waterlogging level, indicating that for the species examined a high uptake of Na^+ did not counteract K^+ uptake. For a given salinity and waterlogging treatment some significant differences were observed between species, presenting always the *Casuarina* species (especially *C. glauca*) higher leaf K^+ concentrations than the other species. Considering all species and treatments, the leaf K^+/Na^+ ratio ranged from 0.07 to 3.5 (data not presented) However there was not a significant correlation between leaf K^+ or K^+/Na^+ ratios and salinity tolerance (ECss_{50}).

Root ion concentrations (Cl^- , Na^+ , K^+)

Differences ($P < 0.01$) were observed between species and salinity treatments in root Cl^- , Na^+ and K^+ concentrations. The waterlogged treatment did not affect ($P > 0.05$) root Cl^- and Na^+ , but increased ($P < 0.05$) root K^+ concentrations by 20 %. The interactions among treatments were not significant ($P > 0.05$), except for “species*salinity” for root Cl^- and Na^+ ($P < 0.01$), and “species*waterlogging” for root Cl^- ($P < 0.01$).

At low salinity ($\text{EC} \sim 2 \text{ dS m}^{-1}$), root Cl^- and Na^+ concentrations were not different ($P > 0.05$) between species (with one exception) in both drained and waterlogged conditions (Table 4). At high salinity ($\text{EC} \sim 17 \text{ dS m}^{-1}$) and drained conditions, the two *Casuarina* species and *E. occidentalis* tended to have higher root Cl^- and Na^+ concentrations than the other species, although the differences were not always significant ($P < 0.05$). Under saline ($\text{EC} \sim 17 \text{ dS m}^{-1}$) and waterlogged conditions, the differences between species in root Cl^- and Na^+ were small and only *A. salicina* showed lower root Na^+ than the rest of species.

Root K^+ concentrations were similar in all species irrespective of saline and waterlogging treatments, and differences among them were not significant ($P > 0.05$) (Table 4).

Discussion

The coupled effects of salinity and waterlogging stresses were more detrimental than either alone in all tested species except in the two *Casuarina* species. This combined negative effect of salinity and hypoxia has been reported also in other tree species including olive (Aragüés et al. 2004) and several Australian tree species (Van der Moezel et al. 1988). Van der Moezel et al. (1989) and Tomar and Gupta (2002) ranked *C. glauca* and *C. obesa* as the most tolerant species to salinity and waterlogging within the *Casuarina* genus. After twelve weeks under the highest level of waterlogged salinity, these were the only two species that fully survived of the six *Casuarina* species analyzed. Patil et al. (1996) in a study with 23 multipurpose trees also ranked *Casuarina* as very suitable for growth under saline and waterlogged conditions. The high salinity tolerance of the two *Casuarina* species found in our study (mean EC_{ss50} values of 21.6 and 19.1 $dS\ m^{-1}$ in drained and waterlogged conditions, respectively) are consistent with works of Marcar et al. (1993, 1999) and Ansari et al (1999), although the last authors found higher tolerance of *A. salicina* compared to *Casuarina*, which differ with our results.

The ratios mean $SDM_{NWT}/mean\ SDM_{WT}$ and mean $RDM_{NWT}/mean\ RDM_{WT}$ were about 0.7 and 0.5, respectively, in the two *Casuarina* species, and higher than 2 and 3, respectively, in the other species. Thus, irrespective of the salinity treatments, the two *Casuarina* species were more tolerant to waterlogging than the other three species. The higher waterlogging tolerance of *Casuarina* has been associated to an increase in the percentage of aerenchyma in the root cortex (Ansari et al., 1999; Van der Moezel 1988, 1989). The importance of constitutive or plastic aerenchymas and their relationships with tolerance to hypoxic conditions has already been established in grassland species (Grimoldi et al. 2005). This mechanism can provide a better energy balance to these species, allowing for a better functioning of the costly leaf salt exclusion mechanisms in waterlogged and saline conditions. This explanation is coherent with the more efficient leaf Cl^- and Na^+ exclusion observed in the two *Casuarina* species (Figs. 4 and 5). The implementation of agroforestry systems using these tolerant *Casuarina* species in salt and waterlogged landscapes would benefit these otherwise unproductive areas.

A. salicina and the two *Eucalyptus* species were less tolerant to salinity than the two *Casuarina* species when they were also subject to waterlogging. *A. salicina* was the most sensitive species to waterlogging irrespective of the salinity treatment, with the highest mean $SDM_{NWT}/mean\ SDM_{WT}$ (3.6) and mean $RDM_{NWT}/mean\ RDM_{WT}$ (7.2) ratios of all tested species. Under non-saline conditions, *E. camaldulensis* showed no significant SDM decreases due to waterlogging, which is consistent with the work of Ansari et al. (1999). *A. salicina* and *E. camaldulensis* were the two most sensitive species to the combination of waterlogging and high salinity ($EC_{ss}\sim 22\ dS\ m^{-1}$) stresses. The low ability of *Eucalyptus* to survive under saline-waterlogged conditions has already been described by Van der Moezel et al. (1991). The higher

($P < 0.05$) salinity tolerance of *E. occidentalis* ($EC_{ss50} = 21.4 \text{ dS m}^{-1}$) than *E. camaldulensis* ($EC_{ss50} = 12.5 \text{ dS m}^{-1}$) under drained conditions (Fig. 2) agrees with the results of Benyon et al (1999) showing 10 % reductions in height growth at EC_e values of 10 (*E. occidentalis*) and 2 dS m^{-1} (*E. camaldulensis*), and tree heights on moderately saline soils of 6.9 m (*E. occidentalis*) and 4.8 m (*E. camaldulensis*).

Under non-saline and waterlogged conditions (2 dS m^{-1} -WT, Fig. 1), *E. camaldulensis* showed similar or higher absolute SDM values than the two *Casuarina* species, but the observed visual symptoms of necrosis at moderate salinity levels (7 dS m^{-1}) and the information provided by other studies suggests that it would be preferable to grow the *Casuarina* species in waterlogged areas. Under non-waterlogging conditions, the two *Casuarina* species showed higher relative salt tolerance than *E. camaldulensis*, which agrees with the previous work of Hussain et al (1994) where *A. salicina* also had higher salinity tolerance than *E. camaldulensis*. A similar result was found by Madsen and Mulligan (2006) in a short-term glasshouse experiment comparing different species and provenances of *Eucalyptus* and *Acacia*. However, the higher rates of leaf Cl^- and Na^+ accumulation in *A. salicina* (Figs. 4 and 5) and the existence of some plants affected by necrosis suggest that in the long term it could be less tolerant to salinity than the two *Eucalyptus* species evaluated.

The salinity tolerance (EC_{ss50}) of the five tree species subject to drained and waterlogged conditions were negatively correlated with their leaf Na^+ ($P < 0.05$) and Cl^- ($P < 0.01$) rates of accumulation per unit increase in EC_{ss} (slopes in Fig. 6). These slopes are similar, indicating that the Na^+ and Cl^- toxicities were the same in these species. These relationships between leaf Na^+ and Cl^- exclusion and salinity tolerance were also found by Van der Moezel et al. (1988, 1989) in different species of *Casuarina* and *Eucalyptus*. Similarly, Aswathappa and Bachelard (1986) found that the two most salt-tolerant *Casuarina* species accumulated less Na^+ and Cl^- in their shoots than the less salt-tolerant species. This exclusion mechanism is particularly important in perennial plants where long times of exposure to salts could exacerbate the accumulation of these toxic ions in the transpiring leaves (Munns and Tester 2008). Thus, Aragüés et al. (2005) found that the salinity tolerance of olive sharply declined after three years of exposure to salts and became quite sensitive due primarily to increasing toxic concentrations of Na^+ in the leaves.

Several studies have shown that leaf K^+ concentrations tend to decrease with increases in salinity in many crops including alfalfa (Isla and Aragüés 2009), horticultural crops (Grattan and Grieve 1999), maize (Isla and Aragüés 2010) and olive (Aragüés et al. 2005). In contrast, in the tested tree species leaf K^+ was not affected by salinity and waterlogging ($P > 0.05$), which also differs from the results obtained by Van der Moezel et al. (1988, 1989) who described a significant decrease in shoot K^+ with salinity in *Casuarina* and *Eucalyptus* species. This discrepancy could be explained by the milder degree of waterlogging in our experiment compared to the Van der Moezel experiments where the water level was established 1 cm above the soil surface. Our milder waterlogging treatment would have allowed the plants to better withstand this stress. Although all the species showed a decrease in leaf Ca^{2+}

concentrations with increases in salinity (Table 2), salinity tolerance and leaf Ca^{2+} were not correlated.

The critical leaf Na^+ and Cl^- concentrations at which trees were severely affected (i.e., high leaf necrosis) or dead by the combination of waterlogging and maximum salinity levels were similar (data not shown). Leaf NaCl concentrations above 1350 meq kg^{-1} resulted in severe leaf necrosis and/or death in *A. salicina*, *E. camaldulensis* and *E. occidentalis*. In contrast, the two *Casuarina* species were less affected because its maximum leaf NaCl concentrations were only 462 (*C. glauca*) and 821 meq kg^{-1} (*C. obesa*) due to its higher ability to exclude these ions from the transpiring leaves. Van der Moezel et al. (1989) also found these relatively low leaf Na^+ and Cl^- concentrations in *C. glauca* and *obesa*, the two most salt and waterlogging tolerant species of the six species tested in their work.

This higher leaf Na^+ and Cl^- exclusion in the two *Casuarina* species examined in our work was related to their ability to restrict the transport of these ions from roots to leaves by partitioning them into roots. Fig. 7 shows that, irrespective of the waterlogging treatment, the salinity tolerance of the five tested species was positively correlated with root Cl^- ($P < 0.05$) and Na^+ ($P = 0.05$) concentrations measured in the 17 dS m^{-1} salinity treatment. This mechanism was also proposed by Chen et al (2002) to explain differences in salinity tolerance in poplar genotypes. Hence, the ability to minimize the transport of these toxic ions to leaves by sequestering them within the roots is a relevant salt-tolerant mechanism for the species tested in this work.

Conclusions

- 1- The salinity tolerance of the five tested tree species (*Acacia salicina*, *Casuarina glauca*, *Casuarina obesa*, *Eucalyptus camaldulensis* and *Eucalyptus occidentalis*) was high, but lower ($P < 0.05$) under waterlogged than under drained conditions. The two *Casuarina* species had the highest plant survival and were the most salt-tolerant species under both drained and waterlogged environments. *E. occidentalis* showed similar salt tolerance as the *Casuarina* species under drained conditions but, irrespective of the salinity level, it was very sensitive to waterlogging. *A. salicina* and *E. camaldulensis* were the two most sensitive species to the combination of waterlogging and high salinity.
- 2- The two *Casuarina* species were highly tolerant to waterlogging, as indicated by their greater shoot and root dry matters under waterlogged than under drained conditions. In contrast, the other three species were sensitive to waterlogging, as indicated by their lower shoot and root dry matters under waterlogged than under drained conditions.
- 3- Leaf Cl^- and Na^+ concentrations increased in all species with increases in soil solution salinity (ECss), and the rate of leaf Cl^- and Na^+ accumulation per unit increase in ECss was much higher under waterlogged than under drained conditions, showing that waterlogging reduced the ability of plants to exclude Cl^- and Na^+ from the leaves.
- 4- The salinity tolerance of the five species depended on the rate of leaf Cl^- and Na^+ accumulation per unit increase in salinity, and this dependency was similar for both ions. The

two *Casuarina* species were less affected by salinity and waterlogging than the other three species due to their higher leaf salt exclusion ability under both drained and waterlogged conditions.

5- The salinity tolerance of the five species was positively correlated with root Na^+ and Cl^- concentrations measured at high salinity, indicating a causal relationship between leaf NaCl exclusion and root NaCl sequestration.

6- Revegetation of saline and waterlogged wastelands with the two most salt and waterlogging tolerant *Casuarina* species is the most profitable strategy for biomass, biofuel and renewable energy production in these otherwise unproductive areas in which the range of suitable species is very limited. The expansion of agroforestry systems using these tolerant species is a sensible strategy that would benefit these saline and waterlogged landscapes.

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Table 1 Average \pm standard deviation electrical conductivity of the irrigation water (ECiw) and the soil solution (ECss) measured in 58 samples collected along the experiment in each target saline treatment. The average Na^+ , Cl^- and Ca^{2+} concentrations and the sodium adsorption ratio (SAR) of the irrigation water are also given

Target saline treatment EC (dS m^{-1})	Irrigation water					Soil solution ECss (dS m^{-1})
	ECiw (dS m^{-1})	Na^+ ----- (meq L^{-1})	Cl^- (meq L^{-1})	Ca^{2+} ----- (meq L^{-1})	SAR [(mmol L^{-1}) $^{-0.5}$]	
2	2.3 ± 0.5	10.0	10.0	7.1	5.0	2.5 ± 0.7
7	6.9 ± 1.6	56.9	53.7	8.0	26.7	7.1 ± 1.4
12	11.6 ± 1.8	108.9	98.3	8.5	49.8	12.2 ± 1.9
17	15.9 ± 2.5	148.7	139.2	8.7	67.3	16.5 ± 2.7
22	20.5 ± 4.0	211.0	182.9	8.8	95.1	21.1 ± 4.2

Table 2 Relationships between leaf ion concentrations (Cl^- , Na^+ and Ca^{2+}) measured at harvest in five species subject to drained (NWT) and waterlogged (WT) treatments, and soil solution salinity (ECss): multiple pairwise comparisons of the slopes of the linear regressions given in the first row. Within each waterlogging treatment, slopes followed by the same letter are not significantly different ($P \geq 0.05$)

Leaf ion concentration (meq kg^{-1}) = Slope \cdot ECss (dS m^{-1}) + Intercept					
Cl^-		Na^+		Ca^{2+}	
Species	Slope	Species	Slope	Species	Slope
NWT (drained treatment)					
<i>C. glauca</i>	(ns) a	<i>C. glauca</i>	7.0 a	<i>E. camaldulensis</i>	(ns) a
<i>C. obesa</i>	(ns) a	<i>E. occidentalis</i>	15.6 ab	<i>C. glauca</i>	-8.1 a
<i>E. occidentalis</i>	14.5 b	<i>C. obesa</i>	16.5 ab	<i>E. occidentalis</i>	-9.8 a
<i>E. camaldulensis</i>	18.8 b	<i>E. camaldulensis</i>	18.4 b	<i>C. obesa</i>	-12.1 ¹ a
<i>A. salicina</i>	38.5 c	<i>A. salicina</i>	48.4 c	<i>A. salicina</i>	-49.6 b
WT (waterlogged treatment)					
<i>C. glauca</i>	15.8 a	<i>C. glauca</i>	18.6 a	<i>E. occidentalis</i>	-4.3 a
<i>C. obesa</i>	34.1 b	<i>C. obesa</i>	40.7 b	<i>C. obesa</i>	-8.5 ab
<i>E. occidentalis</i>	55.8 b	<i>E. occidentalis</i>	51.6 b	<i>E. camaldulensis</i>	-9.7 ab
<i>A. salicina</i>	84.6 c	<i>E. camaldulensis</i>	78.2 c	<i>C. glauca</i>	-10.2 ab
<i>E. camaldulensis</i>	98.5 c	<i>A. salicina</i>	94.1 c	<i>A. salicina</i>	-31.0 b

¹Significantly different from zero at $P = 0.07$

Table 3 Average leaf K⁺ concentrations measured at harvest in four trees of five species subject to three saline (EC = 2, 12, and 22 dS m⁻¹) and two waterlogging (NWT = drained, WT = waterlogged) treatments. Within each salinity and waterlogging treatment, values followed by the same letter are not significantly different ($P \geq 0.05$)

Species	Leaf K ⁺ concentration (meq kg ⁻¹)		
	Saline treatment		
	2 dS m ⁻¹	12 dS m ⁻¹	22 dS m ⁻¹
	NWT (drained treatment)		
<i>A. salicina</i>	169 c	136 c	258 ab
<i>C. glauca</i>	351 a	297 a	299 a
<i>C. obesa</i>	227 b	225 b	231 ab
<i>E. camaldulensis</i>	108 d	155 c	164 b
<i>E. occidentalis</i>	136 cd	126 c	166 b
	WT (waterlogged treatment)		
<i>A. salicina</i>	236 a	202 b	141 ab
<i>C. glauca</i>	250 a	292 a	243 a
<i>C. obesa</i>	195 a	205 b	126 b
<i>E. camaldulensis</i>	137 a	157 b	233 ¹ ab
<i>E. occidentalis</i>	191 a	133 b	142 b

¹ Only one dead plant with attached leaves

Table 4 Average ($n = 4$) root ion concentrations measured at harvest in five species subject to two saline ($EC = 2$ and 17 dS m^{-1}) and two waterlogging (NWT = drained, WT = waterlogged) treatments. Within each salinity and waterlogging treatment, values followed by the same letter are not significantly different ($P \geq 0.05$)

Species	$\text{Cl}^- (\text{meq kg}^{-1})$		$\text{Na}^+ (\text{meq kg}^{-1})$		$\text{K}^+ (\text{meq kg}^{-1})$	
	2 dS m^{-1}	17 dS m^{-1}	2 dS m^{-1}	17 dS m^{-1}	2 dS m^{-1}	17 dS m^{-1}
<u>NWT (drained treatment)</u>						
<i>A. salicina</i>	460 a	922 b	339 b	848 c	199 a	192 a
<i>C. glauca</i>	423 a	1862 a	326 b	1623 ab	97 a	123 a
<i>C. obesa</i>	358 a	1340 ab	313 b	1275 abc	153 a	159 a
<i>E. camaldulensis</i>	367 a	996 b	274 b	992 bc	192 a	184 a
<i>E. occidentalis</i>	482 a	1681 a	496 a	1666 a	182 a	133 a
<u>WT (waterlogged treatment)</u>						
<i>A. salicina</i>	488 a	745 a	387 a	761 b	266 a	212 a
<i>C. glauca</i>	626 a	1379 a	444 a	1388 a	151 a	174 a
<i>C. obesa</i>	505 a	1343 a	500 a	1344 a	174 a	166 a
<i>E. camaldulensis</i>	403 a	922 a	478 a	1074 ab	174 a	212 a
<i>E. occidentalis</i>	403 a	1199 a	500 a	1457 a	199 a	156 a

Figure captions

Fig. 1 Relationships between shoot dry matter (SDM) and soil solution salinity (ECss) in each tree species subject to (a) drained (NWT) and (b) waterlogged (WT) treatments. For each ECss, SDM values with the same letter are not different ($P \geq 0.05$)

Fig. 2 Relationships between relative shoot dry matter and soil solution salinity (ECss) in each tree species subject to drained (NWT) and waterlogged (WT) treatments. The ECss₅₀ estimate, the standard error (SE) and the coefficient of determination (R^2) of the fitted sigmoidal models are also given. Vertical bars indicate \pm one standard error of the mean ($n = 6$)

Fig. 3 Relationships between root dry matter (RDM) and soil solution salinity (ECss) in each tree species subject to drained (NWT) and waterlogged (WT) treatments. Vertical bars indicate \pm one standard error of the mean ($n = 6$)

Fig. 4 Relationships and significant ($P < 0.05$) linear regressions between leaf chloride concentration (leaf Cl^-) and soil solution salinity (ECss) in each tree species subject to drained (NWT) and waterlogged (WT) treatments. The significances of comparisons between the WT and NWT slopes are given in brackets ($*P < 0.05$, $**P < 0.01$). Vertical bars indicate \pm one standard error of the mean ($n = 6$)

Fig. 5 Relationships and significant ($P < 0.05$) linear regressions between leaf sodium concentration (leaf Na^+) and soil solution salinity (ECss) in each tree species subject to drained (NWT) and waterlogged (WT) treatments. The significances of comparisons between the WT and NWT slopes are given in brackets ($*P < 0.05$, $**P < 0.01$). Vertical bars indicate \pm one standard error of the mean ($n = 6$)

Fig. 6 Relationships and linear regressions between the salinity tolerance (ECss₅₀) of the five tree species subject to drained (NWT) and waterlogged (WT) treatments, and the slopes or rates of leaf accumulation of (a) Na^+ and (b) Cl^- per unit increase in ECss. The data for the WT and NWT treatments were pooled together. Legend for species: ¹*C. glauca*, ²*C. obesa*, ³*E. camaldulensis*, ⁴*E. occidentalis*, ⁵*A. salicina*

Fig. 7 Relationships and linear regressions between the salinity tolerance (ECss₅₀) of the five tree species subject to drained (NWT) and waterlogged (WT) treatments, and the root concentrations of (a) Cl^- and (b) Na^+ measured in the saline treatment T17 ($\text{EC} \approx 17 \text{ dS m}^{-1}$). The data for the WT and NWT treatments were pooled together. Legend for species: ¹*C. glauca*, ²*C. obesa*, ³*E. camaldulensis*, ⁴*E. occidentalis*, ⁵*A. salicina*

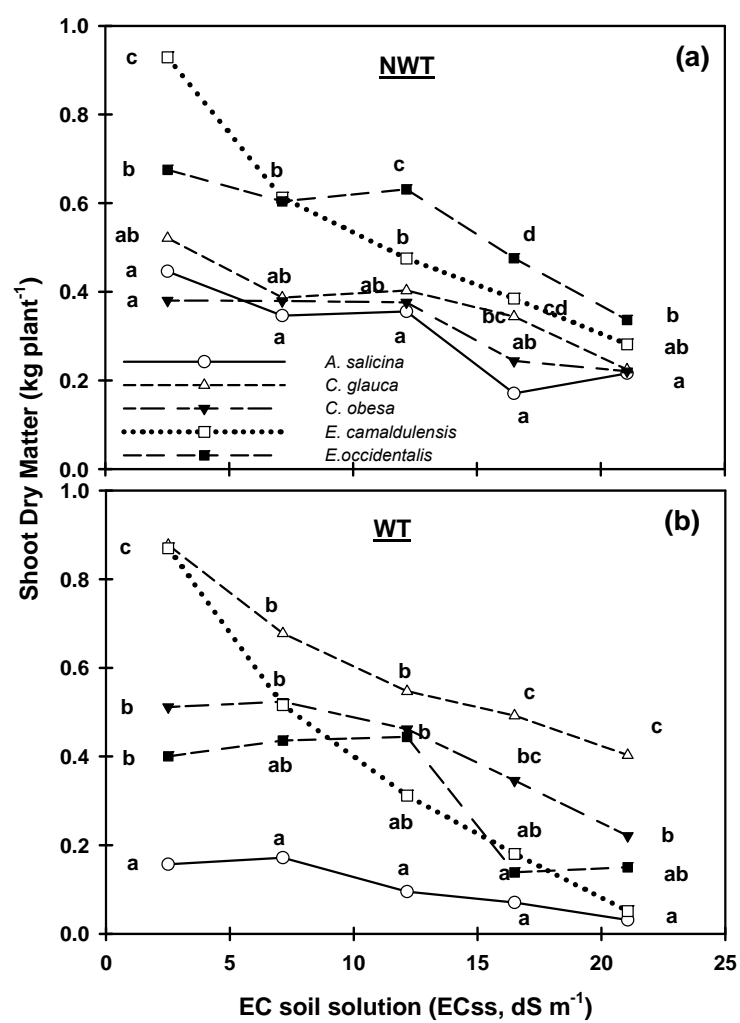


Fig. 1

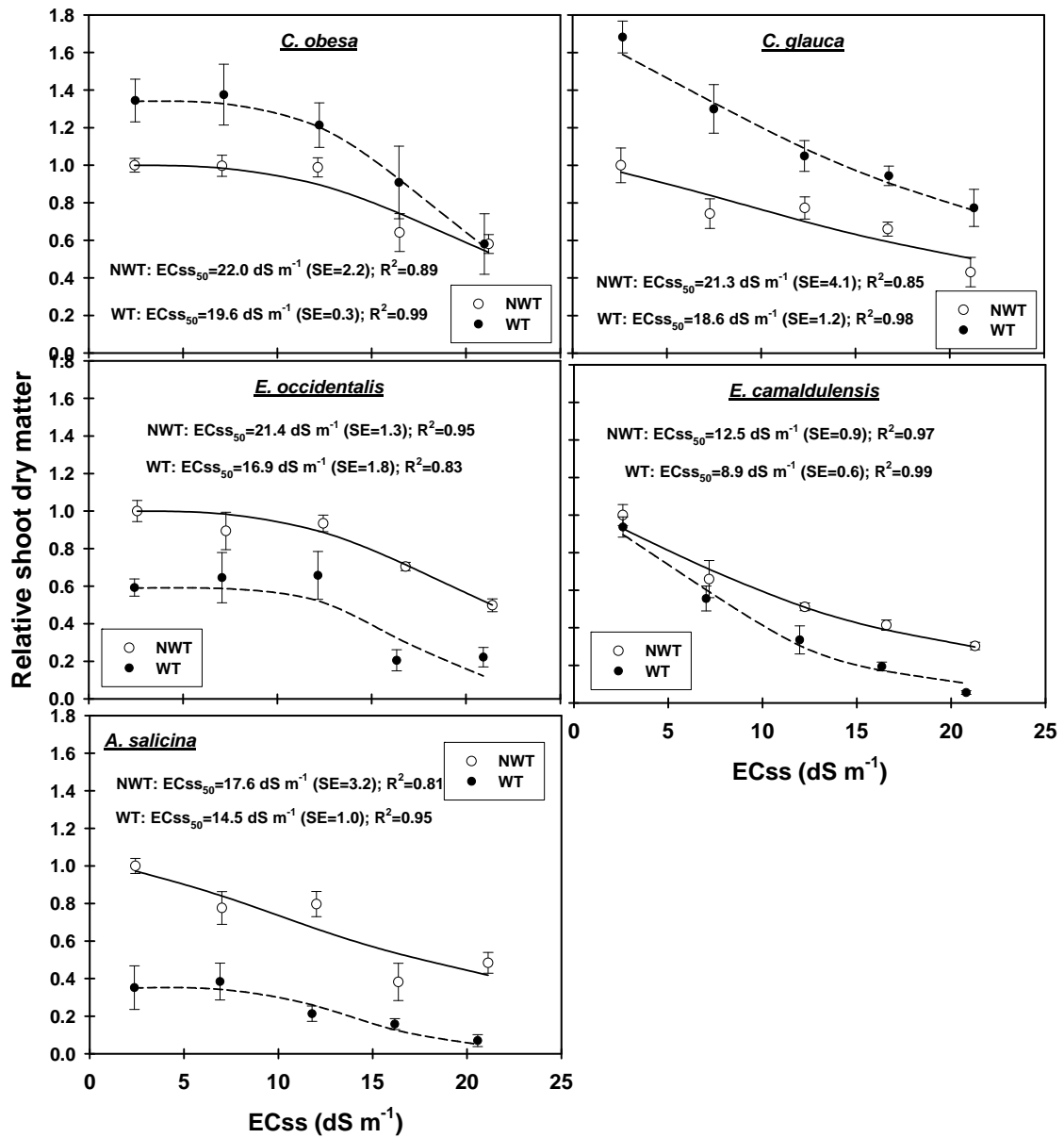


Fig. 2

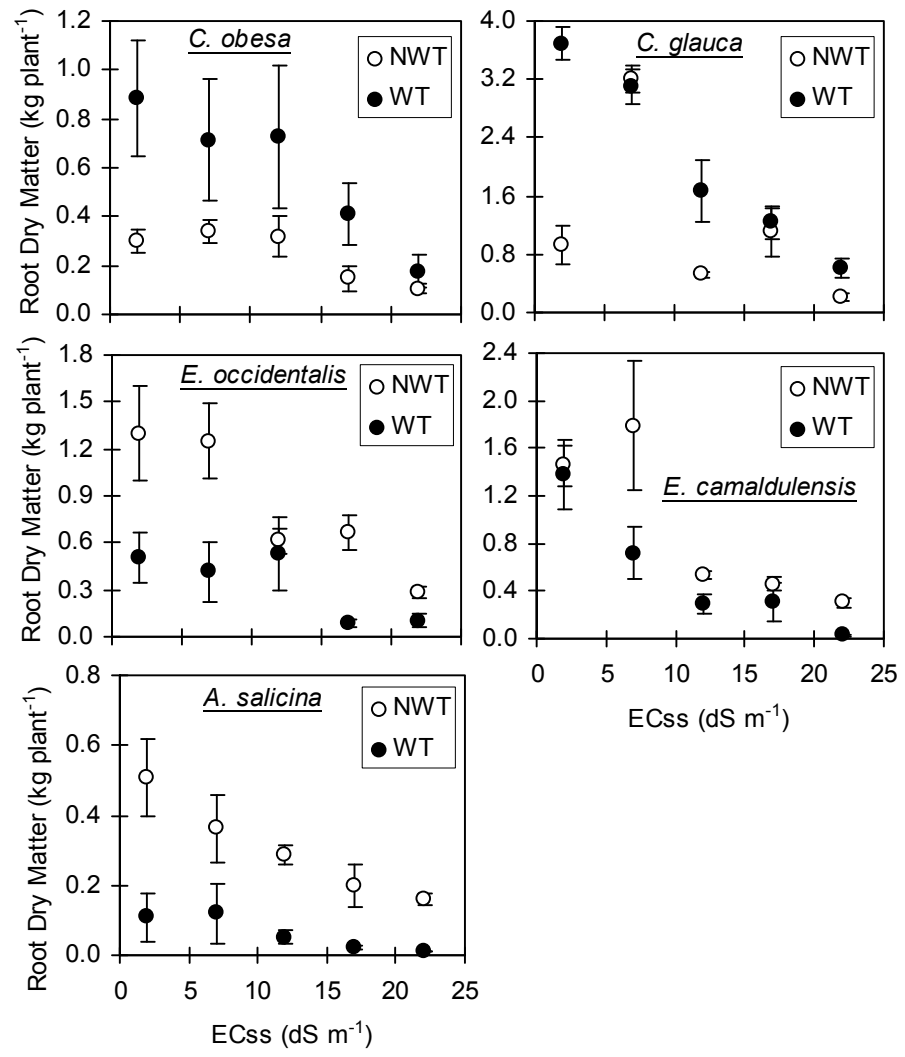


Fig. 3

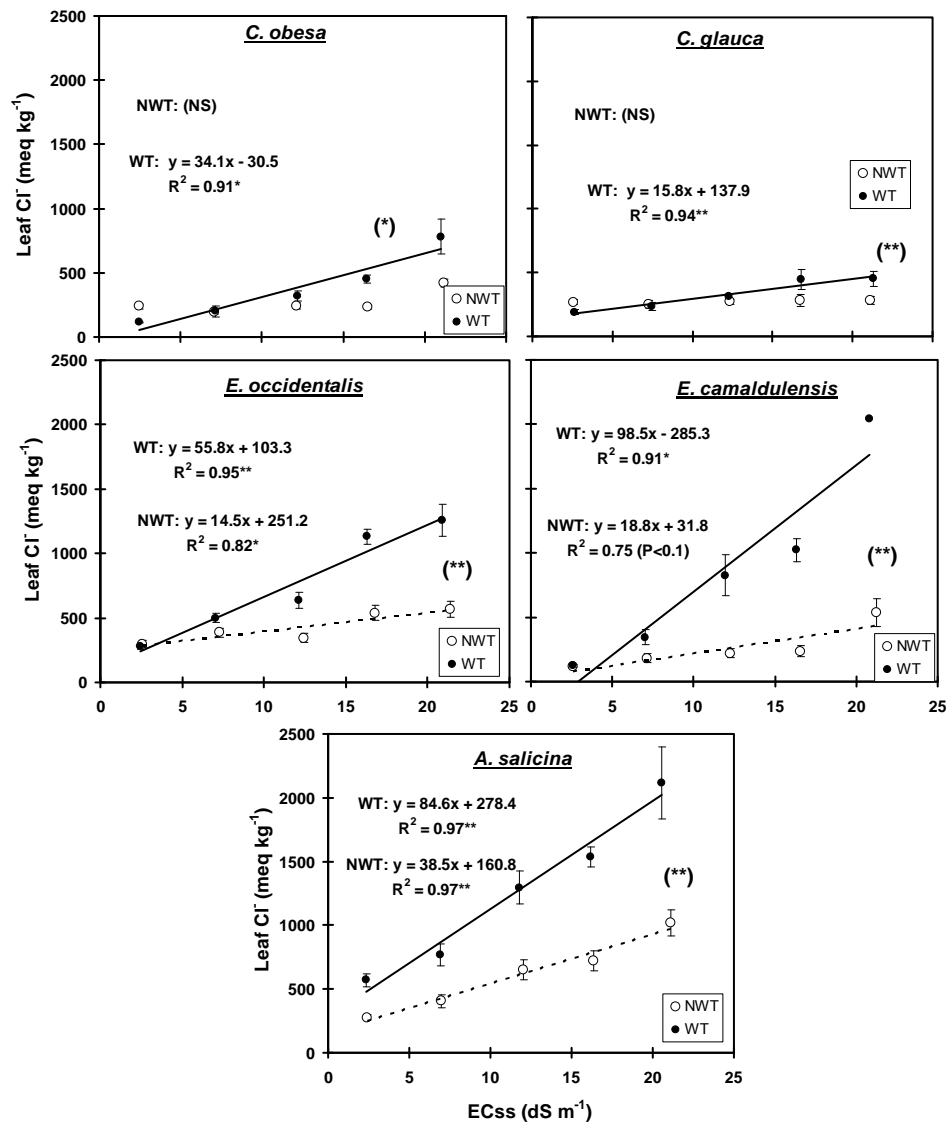


Fig. 4

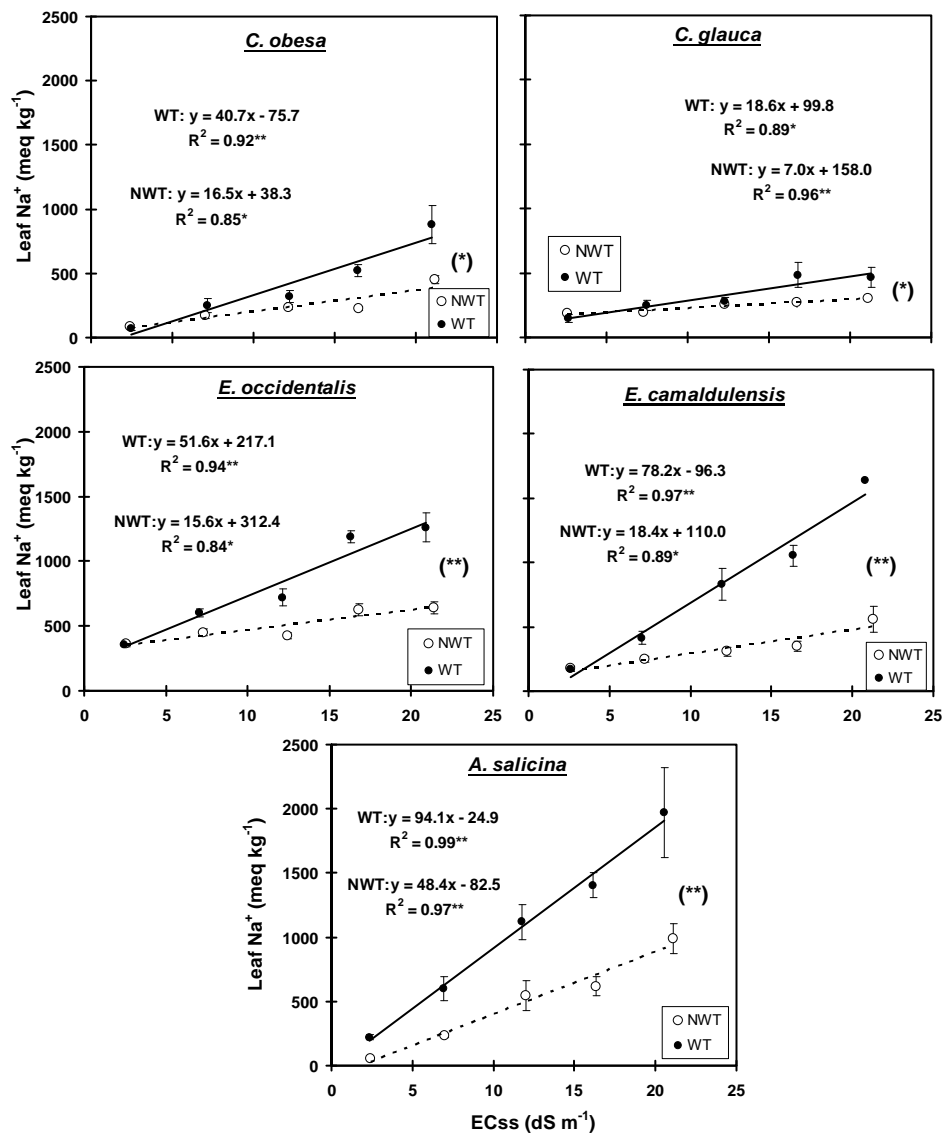


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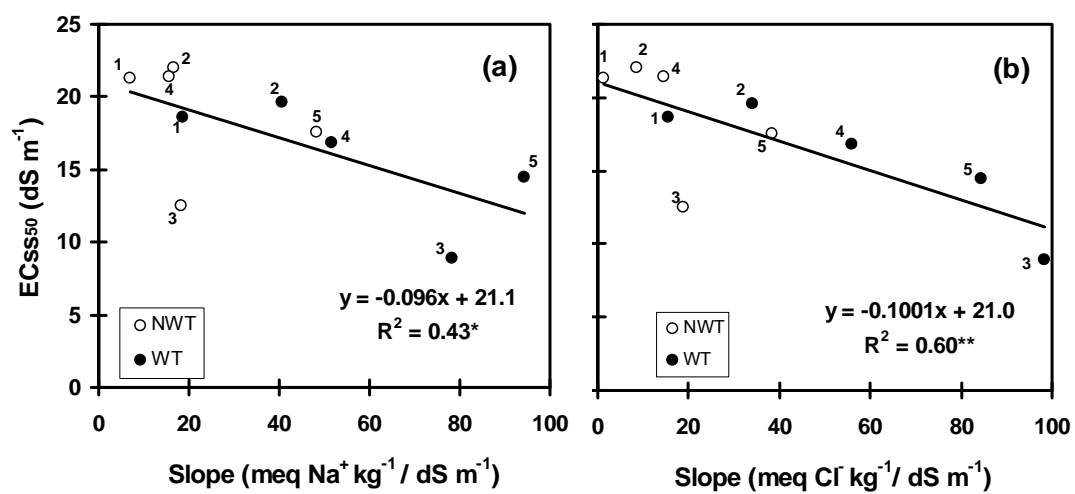


Fig. 6

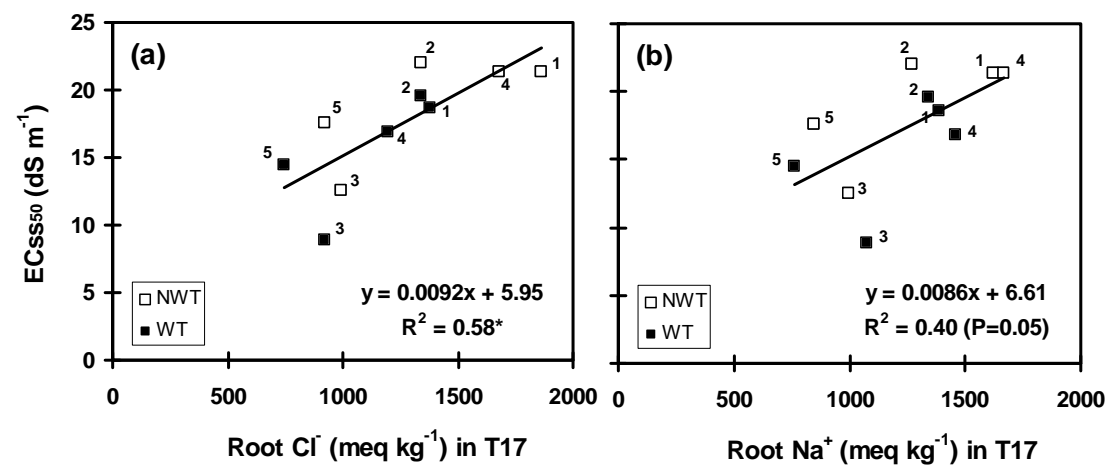


Fig. 7

